DECLARATION OF MAURICIO MIRALLES

I, **Mauricio Miralles**, declare and state as follows:

1. I have been a researcher since 1997 and have a Masters Degree in Molecular Biology.

2. I have been employed by CMH doing genomic research since 2004 and my current position is Genomic Lab Manager. I have held my current position for over 2 years.

3. I have been directly involved with genomic research using probes, including subtelomeric probes as defined in U.S. Patent Application No. 10/676,248("the '248 application") since 2004. Therefore, I would be considered as someone of skill in the art for genomic analysis.

4. This declaration is being submitted to provide information as to how someone of skill would ascertain a sequence given a GenBank Accession Number or a primer as well as addressing the rejection in the Office Action issued on May 27, 2010 regarding the Knight references.

5. The '248 application identifies representative sequences based on their GenBank Accession Number. GenBank Accession Numbers can be easily used to locate the exact sequence and are available on-line. With a GenBank Accession Number one of skill in the art could easily recreate the exact sequence.

6. The '248 application also identifies representative sequences by the primer sequence pairs. Those of skill in the art can easily use these primer pairs to determine the exact sequence referenced in the '248 application. It is well known by those of skill in the art that primer pair sequences generate one and only one sequence. Therefore, if

primer pair sequences were provided to one of skill in the art, they would have the knowledge to use the primer sequences to generate and amplify the sequence and the sequence generated would be identical regardless of who uses the primers to generate the sequence and the lab techniques involved.

7. Those of skill in the art presented with either a GenBank Accession Number or a primer pair sequence would have the knowledge of how to generate and amplify the exact sequence referenced. One of skill in the art would only need either the GenBank Accession Number or the primer pair sequence, not both.

8. The '248 application provides both the GenBank Accession Number and the primer pair sequence for each referenced sequence, therefore, one of skill in the art should have absolutely no issue being able to generate and amplify the exact sequences referenced in the '248 application.

9. In the Office Action issued on May 27, 2010 claims 43 and 45-57 were rejected under 35 U.S.C. 103(a) for allegedly being obvious over Knight (A) et al (Am. J. Human Genetics (2000) volume 67, pages 320-332) in view of Boyle et al (Current Protocols in Molecular Biology (1992) 3.18.1-3.18.9).

10. I have reviewed the Knight (A) and Boyle references and am familiar with their content.

11. It was alleged that Knight (A) taught a method of fluorescence in situ hybridization (FISH) on interphase chromosomes and taught that the probes were labeled and detected. It was further alleged that Knight (A) taught that the distance from the terminal nucleic acid was as little as 268-296 kb for 6ptel48. Knight (A) was alleged to

have taught a method for detecting cytogenetic abnormalities using a plurality of probes within 600 kb of the terminal nucleotide of the chromosome.

12. Knight et al (A) ("Knight (A)") uses BAC probes, which are vectors used to clone DNA fragments and propagated in Escherichia coli cells. Those of skill in the art know that BACs contain large fragments (100 to 300 Kb) of non bacterial DNA. In Knight (A) the non bacterial DNA is derived from a BAC/PAC Library. On page 331, left paragraph, line 1; Knight (A) indicates that the size of the clones range from 100 to 200 Kb.

13. Those of skill in the art know that such large DNA fragments contain repeat sequences. This is confirmed by the other disclosure provided in Knight (A). For example, Knight (A) describes the addition of CoT-1 human DNA. As those of skill in the art are well aware, CoT-1 human DNA is a well known suppressor of –non-specific binding due to repeat sequences presented in probes. If no repeat sequences were present in the reaction, CoT-1 would not be necessary. Knight (A) also uses PCR to screen the clones by sequence tagged sites –STS (page 321, right paragraph, line 4). This allowed Knight to know the relative location of the clones within the telomere region (sequences are shown in Table 2). When we analyzed the adjacent sequences of the STS and expanded up or down stream up to 100 Kb, it is impossible to get a single copy probe (without repeat sequences) that will cover 100 Kb. Fig 1 below shows the location and sequences of clone GS-62-L8. When the sequence is expanded only 10 times (1.2 Kb region) our analysis showed repeat sequences less than 500 bp on either side. Therefore, those of skill in the art would conclude that Knight "clone probes" contain repeat

sequences therein and to avoid cross hybridization with these repeat sequences, they used CoT-1 human DNA.

14. Those of skill in the art would further conclude that the PAC and P1 "probes" of Knight (A) also contained repeat sequences for the same reasons given above for BAC "probes."

16. In contrast, the '248 application uses probes consisting of single copy sequences derived from human DNA. This is reflected in independent claim 43, which reads:

> "A method of screening at least one chromosome of a human for cytogenetic abnormalities, said method comprising the steps of:
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> screening the at least one chromosome of a human using a plurality of single-copy hybridization probes of known human DNA sequence, each of said single copy probes being between 25 bp to about 15 kb in length;
>
> causing said single copy probes to hybridize to the at least one chromosome of the human using a conventional FISH protocol, said hybridization occurring within 600kb of the terminal nucleotide of the at least one chromosome; and
>
> detecting hybridization patterns of said single copy probes, said hybridization patterns indicating cytogenetic abnormalities when they are present in said chromosome."

17. It is my opinion that it would not have been obvious to one of skill in the art to use the disclosure in of Knight (A) to produce a method of screening human

chromosomes using single copy probes from human chromosomes according to the '248 application.

18. Claims 44, 48, 49-52 and 54 were rejected under 35 U.S.C. 103(a) for allegedly being unpatentable over Knight (A) in view of Boyle in view of Knight et al (B) (Journal of Medical Genetics, 2000; 37; 401-409) ("Knight (B)"). It was alleged that Knight (B) taught chromosomal rearrangements involving the telomeres of the chromosomes were emerging as an important cause of human genetic disease s and it was possible to screen for telomeric rearrangements using FISH.

19. I have reviewed Knight (B) and am familiar with its content.

20. Knight (A) in view of Boyle is discussed above. It is my opinion that the probes used in Knight (B) also contain repetitive sequences from human DNA. Knight (B) also uses BAC, PAC, and P1 clones for probes. Claims 44 and 48 depend from claim 43, discussed above, and therefore include the limitation of probes from single copy human sequences. Independent claim 49 also includes this limitation and reads as follows:

> "A method of delineating the extent of a chromosome imbalance in a human individual comprising the steps of:
>
> assaying a subtelomeric region of a human chromosome arm using at least one **single –copy** hybridization probe of known human DNA sequence located within 600 kb of the terminal nucleotide of the chromosome arm, said single copy probe being comprised of human DNA;

hybridizing said single copy probe with said region using a conventional FISH protocol;

detecting hybridization patterns of said single copy probes on said arm; and

comparing said hybridization patterns with a standard genome map of said arm in order to delineate the extent of a chromosome imbalance."

21. Thus, it is clear that both Knight (A) and Knight (B) use probes which include repetitive sequences from human DNA and thus, the probes used in Knight (A) and Knight (B) are differentiated from those of the '248 application.

22. It is also my opinion that Boyle does not make up for the deficiencies in Knight (A).

23. The method of the '248 application would not have been obvious to one of skill in the art in view of Knight (A) in view of Boyle and/or in view of Knight (B).

24. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that those statements were made with the knowledge that willful, false statements and the like are punishable by fine or imprisonment, or both under § 1001 of Title 18 of the United States Code.

Date: 11/23/2010

Mauricio Miralles

Figure 1
GS-62-L8 clone. Coordinates/Sequence

>chr1:1000753-1000873 121bp AGTCTGAAGGTGACAGCGGT AGTGCTCGGAGCCTGGA
AGTCTGAAGGTGACAGCGGTcaggatgcaccttgaactccacccagaagc
cctgcctggccttcccgctggcatcagtggccctgctactcctgcacaca
gcccTCCAGGCTCCGAGCACT

Figure 2